J. Mol. Biol. (1991) **219**, 399–402

Crystallization and Preliminary Diffraction Studies of 5 S rRNA from the Thermophilic Bacterium *Thermus flavus*

Siegfried Lorenz[1], Christian Betzel[2], Elke Raderschall[1], Zbigniew Dauter[2] Keith S. Wilson[2] and Volker A. Erdmann[1]†

[1]*Institut für Biochemie, Freie Universität Berlin Thielallee 63, D-1000 Berlin 33, Germany*

[2]*EMBL c/o DESY, Notkestrasse 85 D-2000 Hamburg 52, Germany*

(Received 16 October 1990; accepted 13 February 1991)

Crystals of purified 5 S rRNA from *Thermus flavus* have been obtained. The crystals diffract up to 8 Å resolution, using synchrotron radiation, and have the monoclinic space-group $C2$. The unit cell has the dimensions $a=190$ Å, $b=110$ Å, $c=138$ Å and $\beta=117°$. The cell volume suggests the presence of four 5 S rRNA molecules per asymmetric unit.

Keywords: 5 S rRNA, crystallization; *Thermus flavus*; 5 S rRNA forms; RNA fast protein liquid chromatography

For the complete understanding of protein biosynthesis the structures of the ribosomes should be known at atomic resolution. Although good progress has been made in crystallizing bacterial ribosomes (Yonath *et al.*, 1980; Yonath & Wittmann, 1989; Hansen *et al.*, 1990), it is clear that their complete X-ray structural analysis will not be accomplished for a long time. It would therefore be of great value in the meantime to determine the structures of ribosomal components. With this goal in mind we have concentrated our efforts on the structure and function of ribosomal 5 S rRNAs, which have been shown by reconstitution techniques to be essential for ribosomal function (Nomura & Erdmann, 1970; Erdmann *et al.*, 1971*a*,*b*; Nierhaus & Dohme, 1974; Dohme & Nierhaus, 1976; Hartmann *et al.*, 1988). Solving the three-dimensional structure of the ribosomal 5 S rRNAs by X-ray diffraction techniques to the highest possible resolution would in addition increase our knowledge of RNA structures in general, since so far only the X-ray structures of several tRNAs (Moras, 1989; Ruff *et al.*, 1988) and the RNA duplex $(U(UA)_6A)_2$ (Dock-Bregeon *et al.*, 1989) have been determined.

Another advantage to studying the 5 S rRNAs is the fact that they interact with several ribosomal proteins to form specific RNA–protein complexes (Horne & Erdmann, 1972). Thus, the structural studies can later be extended to such RNA–protein complex systems in order to increase our knowledge of how proteins recognize, and interact with, ribonucleic acids.

On the basis of chemical and enzymic modification studies (Digweed *et al.*, 1986; Erdmann *et al.*, 1987; Lorenz *et al.*, 1988, 1989) and the alignment of 667 5 S rRNA sequences (Specht *et al.*, 1990), we conclude that the secondary structures of eubacterial, archaebacterial and eukaryotic 5 S rRNAs are known in principle (Specht *et al.*, 1990). The molecules contain five helical regions interrupted by several loops and single-stranded sequences. The 5 S rRNAs are approximately 120 nucleotides long and have a molecular weight of 40,000.

Several attempts have been made in the past to crystallize 5 S rRNAs. Morikawa *et al.* (1982*a*,*b*) reported the first successful crystallization of a 5 S rRNA from *Thermus thermophilus*. Unfortunately the crystals diffracted to a resolution of only 25 Å (1 Å = 0·1 nm). The other report is by Abdel-Meguid *et al.* (1983), who crystallized an *Escherichia coli* 5 S rRNA fragment and its complex with the protein EL-25. Although a resolution of up to 5 Å was reported in the case of the RNA fragment, there has been until now no further detailed structural data available on these studies. From the experience of other laboratories, who have so far solved the structures of only five distinct tRNAs (for a review, see Moras, 1989), we decided to study the 5 S rRNA crystallization by testing the following 18 different 5 S rRNAs species: *Azobacter vinelandii*, *Bacillus licheniformis*, *Bacillus stearothermophilus*, *Bacillus subtilis*, *Caulobacter*, *E. coli*, *Halobacter cutirubrum*, *Micrococcus luteus*, *Proteus vulgaris*, *Pseudomonas fluorescens*, rat liver, *Staphylococcus aureus*,

† Author to whom correspondence should be addressed.

0022–2836/91/110399–04 $03.00/0

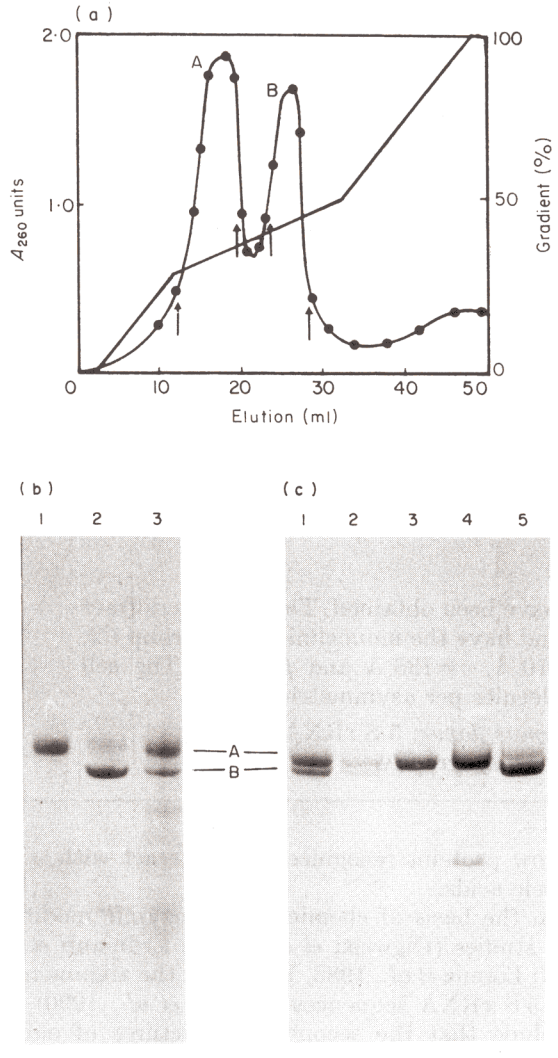


Figure 1. Separation of the 2 5 S rRNA forms A and B from *T. flavus* by (a) fast protein liquid chromatography/ phenyl-Superose column chromatography and ((b) and (c)) denaturing gel electrophoresis. (a) Separation of *T. flavus* 5 S rRNA obtained from Sephadex G-100 chromatography. Peak A corresponds to the 5 S rRNA A form, peak B to the B form. The 5 S rRNAs were eluted by a salt gradient ranging from 1·8 to 0·8 M-ammonium sulphate in 10 mM-ammonium acetate at pH 6·5. (b) Denaturing 5 S rRNA gel electrophoresis. Lane 1: peak A (5 S rRNA A form) from phenyl-Superose column. Lane 2: peak B (5 S rRNA B form) from phenyl-Superose column. Lane 3: 5 S rRNA mixture applied to phenyl-Superose column. (c) Denaturing gel electrophoresis of different 5 S rRNA materials before and after crystallizations. The denaturing gel electrophoresis conditions were as follows. Gel: 9·5% (w/v) acrylamide, 0·5% (w/v) *N*, *N*-methelenebisacrylamide, 7 M-urea, 90 mM-Tris-(hydroxymethyl)-ammonium methane, 90 mM-boric acid, 2·5 mM-EDTA (pH 8·4). Buffer: 90 mM-Tris-(hydroxymethyl)-ammonium methane, 90 mM-boric acid, 2·5 mM-EDTA (pH 8·4). Lane 1: 5 S rRNA fraction obtained from Sephadex G-100 chromatography as used in crystallization experiments. Lane 2: electrophoresis of 1 dissolved crystal obtained from the material analysed in lane 1. Lane 3: a form of 5 S rRNA after isolation on phenyl-Superose and prior to crystallization. Lane 4: 5 S rRNA as isolated from a crystal obtained from material analysed in lane 3. Lane 5: B form of 5 S rRNA after isolation on phenyl-Superose and prior to crystallization. The B form gave only microcrystals under the conditions studied.

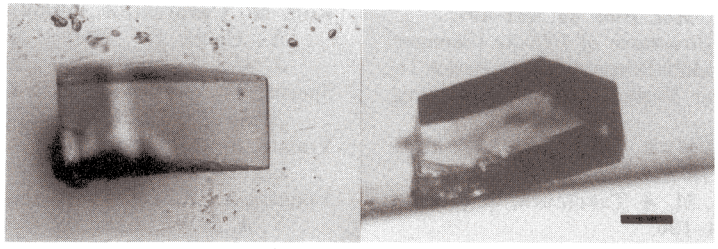
Thermotoga, *Thermus aquaticus*, *Thermus flavus*, *Thermus thermophilus*, wheat germ and yeast. So far the 5 S rRNA from *Thermus flavus* has yielded the best crystals, so we report these results.

Cells from *T. flavus* strain AT-62 were grown at 75 °C. The 70 S and 50 S ribosomal subunits were isolated as described by Cronenberger & Erdmann (1975). The 5 S rRNA was prepared from 70 S ribosomes or 50 S ribosomal subunits by extraction with phenol (Erdmann *et al.*, 1971*a*,*b*) and purified by two successive chromatographic procedures on Sephadex G-100. Since gel electrophoretic analysis revealed the presence of two 5 S rRNA forms in the 5 S rRNA preparations, the A and B forms, we separated these two forms by hydrophobic interaction chromatography on a phenyl-Superose column (Fig. 1). It is of interest that there are no sequence differences between the A and B forms of the 5 S rRNA (data not shown). The A and B forms observed for the *T. flavus* 5 S rRNA are different from those described earlier for *E. coli* 5 S rRNA (Aubert *et al.*, 1968; Digweed *et al.*, 1982), since we were unable to convert, under several different sets of conditions, one form into the other.

From various crystallization methods tested we found that the microdialysis method first described by Zeppezauer *et al.* (1968), was best suited for 5 S rRNA crystallization. Crystallization was attempted routinely at 4 and 18 °C, and several typical crystals obtained are shown in Figure 2.

Gel electrophoresis of the materials used for crystallization and of the materials found in the crystals showed that a mixture of the A and B forms, and the A form by itself, are crystallizable (Figs 1 and 2). The results presented in Figure 1 show in addition that the A and B forms, when present in the starting material, are able to co-crystallize. The B form by itself gave only microcrystals (data not shown). X-ray analysis of the crystals revealed that the highest resolution was obtained with crystals containing only the A form of the 5 S rRNA preparation.

The ribosomal 5 S RNA crystals were first characterized using the image plate scanner on the X-11 synchrotron beamline in the EMBL Outstation, at the DORIS storage ring, DESY, Hamburg. The DORIS ring operated in a parasitic mode. Several crystals were mounted in thin-walled glass capillaries, 0·5 mm or 0·7 mm in diameter. From one crystal several rotation images were recorded using a wavelength of 0·965 Å and a crystal-to-plate distance of 500 mm. The plate has a diameter of 220 mm. The oscillation range per exposure was 1°, and exposure times were about 200 seconds per image. The read-out time for each image was about 100 seconds. The diffraction extends up to 8 Å resolution (Fig. 3). After transformation to Cartesain co-ordinates and correction for non-uniformity, the images were



(a) (b)

Figure 2. Typical crystals from *T. flavus* 5 S rRNA grown under the conditions described in the legend to Fig. 1. (a) A+B form. (b) A form. 5 S rRNA solutions (1 to 2 mg/ml) in sample buffer (15 mM-sodium cacodylate (pH 7·0), 30 mM-NaCl, 1 mM-spermine, 15 mM-magnesium chloride and 20% saturated ammonium sulphate) were dialysed against the same buffer, but with a contents of 40 to 60% saturated ammonium sulphate. After approximately 1 month, stable and well-shaped single crystals up to 0·45 mm × 0·2 mm × 0·2 mm in size were obtained. The bar represents 0·1 mm.

evaluated using a modified version of the MOSFLM film integration package (Leslie *et al.*, 1986).

The preliminary assignment of the *T. flavus* 5 S rRNA crystal lattice is monoclinic, space-group *C*2, with unit cell dimensions $a=190$ Å, $b=110$ Å, $c=138$ Å and $\beta=117°$. The unit cell volume is $2{\cdot}69\times10^6$ Å. Assuming that the 5 S rRNA molecules have a mol. mass of 160,000 per asymmetric unit, the value of the packing volume is 4·3 Å^3/dalton, which is higher than the range usually observed for protein crystals (1·7 to 3·5 Å^3/dalton; Matthews, 1968), but has been observed for crystals of tRNAs. This gives a solvent content for the crystals of 70%. All images used in characterizing the cell were recorded at room temperature from a single crystal, without indication of major radiation damage. From the results presented we conclude that the ribosomal 5 S RNA can eventually be crystallized to determine its atomic structure. We are determined to continue these studies by trying other 5 S rRNA species and different isolation procedures until this goal has been reached.

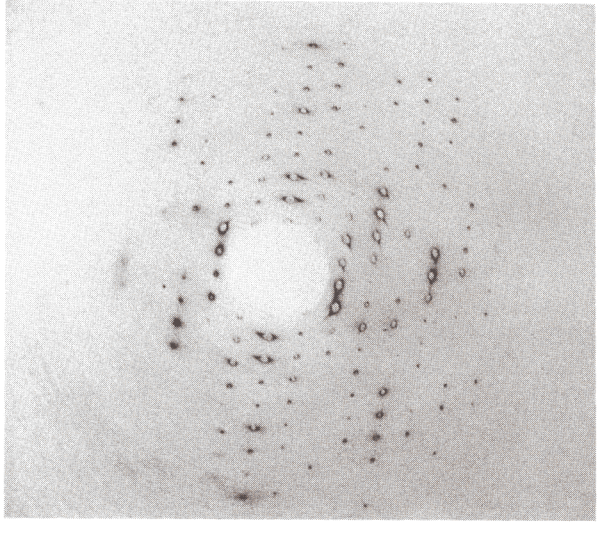


Figure 3. Oscillation image of the A form of 5 S rRNA recorded with the image plate scanner. The image has been photographed from the screen of a micro-VAX-II GPX workstation. The resolution obtained is about 8 Å.

The authors thank the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie. We also thank Christine Kansy for typing the manuscript and Angela Schreiber for the photographs.

References

Abdel-Meguid, S. S., Moore, P. B. & Steitz, T. A. (1983). *J. Mol. Biol.* **171**, 207–215.

Aubert, M., Scott, J. F., Reynier, M. & Monier, M. (1968). *Proc. Nat. Acad. Sci., U.S.A.* **61**, 292–299.

Cronenberger, G. H. & Erdmann, V. A. (1975). *J. Mol. Biol.* **95**, 125–137.

Digweed, M., Kumagai, I., Pieler, T. & Erdmann, V. A. (1982). *Eur. J. Biochem.* **127**, 531–537.

Digweed, M., Pieler, T. & Erdmann, V. A. (1986). *Structure and Dynamics of RNA* (van Knippenberg, P. H. & Hilbers, C. W., eds), pp. 205–219, Plenum, New York.

Dock-Bregeon, A. C., Chevrier, B., Podjarny, A., Johnson, J., de Bear, J. S., Gough, G. R., Gilham, P. T. & Moras, D. (1989). *J. Mol. Biol.* **209**, 459–474.

Dohme, F. & Nierhaus, K. M. (1976). *J. Mol. Biol.* **107**, 585–595.

Erdmann, V. A., Fahnestock, S., Higo, K. & Nomura, M. (1971*a*). *Proc. Nat. Acad. Sci., U.S.A.* **68**, 2932–2936.

Erdmann, V. A., Doberer, H. G. & Sprinzl, M. (1971*b*). *Mol. Gen. Genet.* **114**, 89–94.

Erdmann, V. A., Wolters, J., Pieler, T., Digweed, M., Specht, T. & Ulbrich, N. (1987). *Ann. N.Y. Acad. Sci.* **503**, 103–124.

Hansen, H. A. S., Volkmann, N., Piefke, J., Glotz, C., Weinstein, S., Makowki, I., Meyer, S., Wittmann, H. G. & Yonath, A. (1990). *Biochim. Biophys. Acta*, in the press.

Hartmann, R. K., Vogel, D. W., Walker, R. T. & Erdmann, V. A. (1988). *Nucl. Acids Res.* **16**, 3511–3524.

Horne, J. R. & Erdmann, V. A. (1972). *Mol. Gen. Genet* **119**, 337–344.

Leslie, A. G. W., Brick, P. & Wonacott, A. J. (1986). *CCP4 Newsletters* **18**, 33–39.

Lorenz, S., Hartmann, R. K., Specht, T., Ulbrich, N. & Erdmann, V. A. (1988). *Mol. Cell. Biol. (Life Sci. Advan.)* **7**, 65–73.

Lorenz, S., Hartmann, R. K., Schultze, S., Ulbrich, N. & Erdmann, V. A. (1989). *Biochemie*, **71**, 1185–1191.

Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Moras, D. (1989). *Crystal Structures of tRNAs* (Saenger, W. ed.), pp. 1–30, Landolt-Börnstein New Series 1b, Nucleic Acids, Springer Verlag, Berlin, Heidelberg, and New York.
Morikawa, K., Kawakami, M. & Takemura, S. (1982*a*). *Nucl. Acids Res.* **11**, 173–176.
Morikawa, K., Kawakami, M. & Takemura, S. (1982*b*). *FEBS Letters* **145**, 194–196.
Nierhaus, K. M. & Dohme, F. (1974). *Proc. Nat. Acad. Sci., U.S.A.* **71**, 4713–4717.
Nomura, M. & Erdmann, V. A. (1970). *Nature (London)*, **228**, 744–748.
Ruff, M., Cavarelli, J., Mikol, V., Lorber, B., Mitschler, A., Giege, R., Thierry, J. C. & Moras, D. (1988). *J. Mol. Biol.* **201**, 235–236.
Specht, T., Wolters, J. & Erdmann, V. A. (1990). *Nucl. Acids Res.* **18** (suppl.), 2215–2230.
Yonath, A. & Wittmann, H. G. (1989). *Trends Biochem. Sci.* **14**, 329–335.
Yonath, A., Müssig, J., Tesche, B., Lorenz, S., Erdmann, V. A. & Wittmann, H. G. (1980). *Biochem. Intern.* **1**, 428–435.
Zeppezauer, A. G. W., Eklund, H. & Zeppezauer, E. (1968). *Arch. Biochem. Biophys.* **126**, 564.

Edited by R. Huber